Title: Sickle Cell Disease *GeneReview* Hemoglobin Assays: Advantages and Disadvantages
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Hemoglobin Assays: Advantages and Disadvantages

High-performance liquid chromatography (HPLC)

- Readily separates some proteins that cannot be resolved by other means;
- Allows for accurate quantification of normal and variant hemoglobins at low concentrations, enabling differentiation of Hb S/β^+-thalassemia from sickle cell trait (Hb A/S), as well as a quantitative description of compound heterozygous disorders such as Hb S/HPFH (hereditary persistence of fetal hemoglobin; see Beta-Thalassemia, Genotype-Phenotype Correlations) and Hb S/C;
- Does not definitively distinguish Hb S/β°-thalassemia from Hb S/S (identification requires DNA testing or integration with other laboratory studies).

Isoelectric focusing

- Capable of higher resolution than other hemoglobin electrophoresis
- Routine isoelectric focusing provides an efficient platform for high-throughput screening and thus is often used for newborn screening, but is less quantitative than HPLC.
- Capillary isoelectric focusing technology allows for separation of very small samples, quantification, and automation of sampling.

Cellulose acetate electrophoresis and citrate agar electrophoresis

- Useful for quick screening of a small number of samples
- Protein bands are relatively wide and many abnormal hemoglobins overlap.
- Quantitative densitometry of abnormal hemoglobins is inaccurate at low concentrations (e.g., HbA2 and HbF in adults).

Kleihauer-Betke test

- Acid-elution test that detects the presence of cells with high fetal hemoglobin content.
- Can be used to characterize coexistent hereditary persistence of fetal hemoglobin (HPFH) with SCD.

Solubility test (i.e., Sickledex, Sickleprep, or Sicklequik)

- Utilizes the relative insolubility of deoxygenated HbS in solutions of high molarity. Hemoglobin S in hemolysates precipitate in the test solution while other hemoglobins remain in solution.

- Solubility tests alone should NEVER be used for genetic counseling assessments if one partner is known to have HbS, as this test will not detect β°-thalassemia, HbC, or other hemoglobin variants that can lead to compound heterozygous forms of SCD.

  The solubility test has no place in the definitive diagnosis of SCD because:

  - It does not differentiate SCD from sickle cell trait (Hb A/S);
  - False positives have been reported [Hara 1973];
  - High levels of HbF may cause false negative results in infants with SCD;
  - It may miss some clinically significant forms of sickle hemoglobinopathies (e.g., Hb S/C) [Fabry et al 2003].

  The main uses for the solubility tests are:

  - A low-cost and rapid screen for the presence of HbS prior to investing in definitive testing; and
  - Emergent estimation of whether a clinically significant hemoglobinopathy exists (if combined with a CBC, blood smear, and reticulocyte count).

References

Fabry ME, Archyra SA, Suzuka SM, Nagel RL. Solubility measurement of the sickle polymer. In: Nagel RL, ed. *Hemoglobin Disorders: Molecular Methods and Protocols*. Totowa, NJ: Humana Press; 2003:271-87.

Hara S. Reliability and modification of Sickledex test. J Natl Med Assoc. 1973;1973;65:431.